6-2005

Psychological Stress and High Sensitivity C-Reactive Protein Levels in Overweight and Obese Men

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PSYCHOLOGICAL STRESS AND HIGH SENSITIVITY C-REACTIVE PROTEIN LEVELS IN OVERWEIGHT AND OBESE MEN

by

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A Dissertation in Partial Fulfillment

of the Requirements for the

Degree of Doctor of Public Health

in Preventive Care

June 2005

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ABSTRACT OF DISSERTATION

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Loma Linda University, Loma Linda University, 2005

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Background and Purpose. High sensitivity C-reactive protein (hs-CRP), a measure of inflammatory response, is now considered an independent marker for coronary heart disease. Psychological stress also affects the immune system and influences the inflammatory response. Ladwig, et al (2003) found a significant positive association between hs-CRP levels and depression ($F=4.9$, $p=.008$) in 726 obese males, even after adjusting for smoking, high blood pressure and age. This study investigated if there was a similar association between increased hs-CRP levels and psychological stress in overweight and obese males.

Method. Sixty-one overweight (n=28) or obese (n= 33) males, ages 20-35, were recruited to participate in a cross-sectional study. Variables assessed were body mass index (BMI), body fat percentage, blood pressure, perceived stress using Cohen et al's (1983) Perceived Stress Scale (PSS), and hs-CRP blood levels.

Results. There was no linear association between PSS score and hs-CRP (Spearman's rho r=.06, p=.65). Only 11 (18.0%) subjects had hs-CRP levels above 3, the level considered to be high risk, while almost half (n=28, 45.9%) had levels less than 1. Statistically significant Spearman's rho correlations were found between hs-CRP and BMI (r=.55, p=.00001), hs-CRP and body fat percentage (r=.59, p=.000001), and a trend for perceived stress score and BMI (r=.23, p=.07). Levels of hs-CRP were higher in obese than overweight subjects (mean = 2.7 vs 0.9, p=.00003).

Conclusion. This study confirmed an association of hs-CRP with BMI and body fat but showed no linear relationship of chronic stress of daily life to hs-CRP levels.

Importance to Preventive Care. Although this study did not find an association between chronic stress and hs-CRP, previous research indicates that acute stressors do play a role in increasing this measure of inflammatory response. Preventive care specialists need to consider reducing obesity and possibly accounting for acute, rather than chronic, stressors when prescribing lifestyle changes needed for optimal health.

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ACKNOWLEDGEMENTS

I want to thank my parents, Bhagyam and Franklin Moses for their support and sacrifice, which has allowed me to have this experience. You have provided me not only with spiritual tools but professional tools that I will cherish and use for the rest of my life. The example you have provided by your life has not only been my foundation but something to emulate.

I would also like to thank my sister Stephanie Moses for volunteering her clinical laboratory science expertise. You have been my cheerleader and defender my entire life which continued on throughout this graduate school experience. Thank you from the bottom of my heart.

I am forever grateful to my committee members Dr. Hellen Hopp Marshak and Dr. Brenda Rea for their support and guidance throughout this dissertation experience. Your professionalism, knowledge, hard work and dedication have truly propelled me through this process.

CHAPTER 1

INTRODUCTION

A. Statement of the Problem

The rate of overweight and obese individuals has been increasing at a phenomenal rate over the last two decades. According to the Centers for Disease Control and Prevention (CDCP) (2003), the percentage of obese adults has increased from 12.0% in 1991 to 20.9% in 2001. Results from the National Health and Nutrition Examination Survey (NHANES) show that in 1999-2000, an estimated 64% of U.S. adults were either overweight or obese (CDCP, 2004). This translates into millions of people suffering from extra weight and the many health consequences that overweight and obesity produce. It is estimated that in 2002, national costs attributed to both overweight and obesity accounted for medical expenditures of $9.2 billion (Centers for Disease Control and Prevention, 2002). The United States is experiencing an overweight and obesity epidemic that warrants special attention.

Heart disease, whether it is called coronary artery disease (CAD) or cardiovascular disease (CVD), is another negative health condition that can have devastating effects on well-being. Heart disease is one of the many health consequences associated with overweight and obesity (Hiroshi, et al., 2004; Matsuzawa, Shimomura, Kihara & Funahashi, 2003; Watkins, 2004). Currently, CAD is one of the leading causes of death in the United States with nearly 40% of all deaths associated with heart disease and stroke. Over 930,000 Americans die of cardiovascular disease a year which translates into one death every 34 seconds (CDCP, 2004). Despite the use of new

pharmacologic and lifestyle approaches to lower plasma cholesterol concentrations, cardiovascular disease continues to be the main cause of death in the United States, Europe, and much of Asia (Ross, 1999).

Lifestyle interventions and prevention strategies are a vital aspect of CAD treatment and prevention as well as other diseases such as diabetes (Mosca, 2002; Sahay & Sahay, 2002; Sdringola, et al, 2003). Lifestyle changes such as improving diet, weight loss and smoking cessation lowers disease risk. Therefore these strategies have been the major focus of prevention interventions.

Stress reduction is likely to become another major target for lifestyle intervention of CAD in the future. Studies show that psychological states such as depression are associated with increased biomarkers of inflammation which are linked to CAD (Black, 2003; Dugue, Leppaen, Teppo, Fyhrquist & Grasbeck, 1993). Inflammatory cytokines such as interleukin-6, tumor necrosis factor (TNF), and C-reactive protein are among the biomarkers noted in the association between stress and inflammation. Many studies have shown a mind-body connection, specifically with psychological states such as depression and stress and their influence on bodily reactions such as their effects on the immune and inflammatory systems (Dugue, Leppaen, Teppo, Fyhrquist & Grasbeck, 1993; Hapauarachchi, Chalmers, Winefield & Blake-Mortimer, 2003; Ladwig, Marten-Mittag, Lowel, Doring & Koenig, 2003; Lesperance, Frasure-Smith, Theroux & Irwin, 2004).

In 2003, the American Heart Association and the Centers for Disease Control and Prevention published a joint scientific statement in *Circulation*, stating that from a pathological viewpoint, all stages of atherosclerosis, which include initiation, growth, and complication of atherosclerotic plaque, might now be considered to be an inflammatory

issue (Person, et al., 2003). This redirects the idea that atherosclerotic plaque is primarily due to excess LDL cholesterol level in the blood. A recent study found that C-reactive protein (CRP) level is a better predictor of cardiovascular events than the LDL cholesterol level (Ridker, 2002).

Many things, such as injury and pathogen invasion, can trigger the process of inflammation resulting in CRP production (Dovi, Szpaderska & DiPietro, 2004; Sumi, Satoh, Ishikawa & Sekizawa, 2004). However, studies show that psychological factors, such as depression, stress and terror can also induce an inflammatory response (Lesperance, Frasure-Smith, Theroux & Irwin, 2004; Hapauarachchi, Chalmers, Winefield & Blake-Mortimer, 2003; Melamed, Shirom, Toker, Berliner & Shapira, 2004). In particular, stress is seen to activate the acute phase response, which is part of the innate immune inflammatory response (Black, 2003). Based on this association, psychological stress may be a focus area for intervention in the future for preventive measures targeting heart disease risk and risk of future cardiac events.

Other than obesity, there are no specific physical findings that show a correlation with the body and general CRP levels. However, many patient conditions and characteristics, such as hyperglycemia, metabolic syndrome, chronic infection, weight loss, medication and alcohol consumption, are associated with increased or decreased levels of CRP using the high sensitivity CRP (hs-CRP) test (King, Mainous & Taylor, 2004). High sensitivity CRP testing (hs-CRP) is used in the detection of atherosclerosis because atherosclerosis is now being considered an inflammatory disease (Ladwig, Marten-Mittag, Lowel, Doring & Koenig, 2003; Ridker, Fifai, Rose, Buring & Cook, 2002; Ferranti & Rifai, 2002). The initiation of an inflammatory response can be

detected by examining liver activity. Liver cells, also known as hepatocytes, are very active in the production of acute-phase reactants, which are used as biomarkers for inflammation. Acute phase reactants are a diverse group of proteins regularly seen during acute and chronic systemic inflammation reactions. These proteins are part of the host's defense mechanism against tissue damage and infection. During acute, systemic inflammatory diseases, hormonal mediators such as interleukin-6, tumor necrosis factor-α (TNF- α), glucocorticoids, and interferon-γ are produced in the liver (Feldman: Sleisenger & Fordtran's Gastrointestinal and Liver Disease, 2002). Interleukin-6 and tumor necrosis factor-α are major modulators of the inflammation biomarker C-reactive protein (CRP). C-reactive protein is a pentamer consisting of five identical, noncovalently linked, 23-kD subunit produced in the liver at increased levels during inflammatory states (Ruddy, 2001). Elevations of inflammatory mediators may contribute to the pathogenesis of atherosclerosis, because atherosclerosis is actually the inflammation of the arterial wall (Dandona & Aljada, 2002).

When the body experiences a physical invasion or imbalance, inflammation is triggered. For example, high levels of CRP are found in individuals with acute bacterial infections, major trauma, systemic vasculitis and viral infections. It is now clear, due to recent studies, that psychological issues may be added to the list of factors that influence elevated hs-CRP levels (Ruddy, 2001). Ladwig, Marten-Mittag, Lowel, Doring and Koenig (2003) studied the relationship of depressive mood to high sensitivity C-reactive protein (hs-CRP) in 3,205 males, of which 726 (23%) were obese. Although no association of hs-CRP and depression across all subjects was found there was a clear association of elevated CRP levels and depression in obese males ($F=4.9$, $p=.008$). These

results indicate that obesity and mental state may interact to elevate hs-CRP levels. Although the Ladwig, et al study cannot determine causality, obesity may have a synergistic relationship with psychological factors that may ultimately lead to disease states or damage to the body.

The problems of obesity and CAD can have devastating impact on general health and quality of life (Neilson & Schneider, 2005). Intervention strategies such as lowering weight and total and LDL cholesterol levels, are used to combat these conditions. These practices are important to reducing risk of cardiac events due to CAD; however, more research is needed to uncover other variables, such as psychological factors, that can be targeted to enhance prevention and treatment strategies for heart disease.

The concept that the mind can activate an inflammatory response is important because of its implications for CAD. Research that examines the possible association of obesity, psychological factors and inflammation may lead to the identification of new intervention points for CAD prevention. The present study examined the idea that psychological state is associated with inflammation among overweight and obese males, as indicated by hs-CRP levels, which in turn is related to increased risk of CAD. Therefore, the association of stress and hs-CRP levels in overweight and obese individuals may increase the risk of CAD.

Therefore, the present study investigated a relationship, as depicted in Figure 1, of how stress level might influence hs-CRP levels in the blood, thereby increasing atherosclerotic risk in overweight or obese males. Ladwig, et al. (2003) suggested a similar relationship using depression as the psychological state in their study.

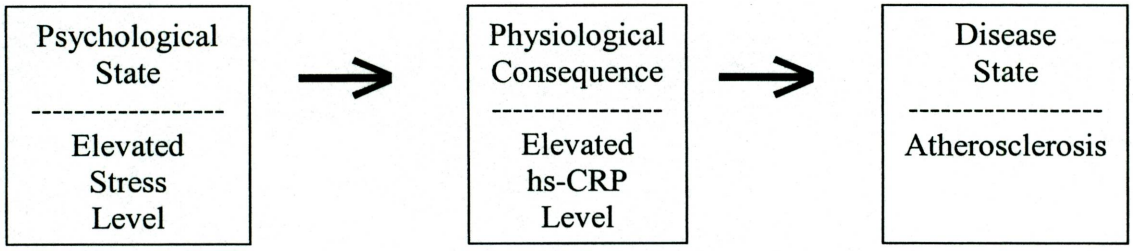

Figure 1. Proposed pathway of events related to stress, hs-CRP and atherosclerosis adapted from Ladwig, et al (2003). *(Elevated stress level increases level of hs-CRP resulting in atherosclerosis.)*

B. Purpose of the Study

Ladwig, et al., studied the relationship of depressive mood to CRP in obese men. Psychological states other than depressive mood may be associated with increased CRP levels in obese and overweight individuals. Using the Ladwig, et al. study as a template, the present study examined stress instead of depressive mood as the psychological state that potentially triggers elevated hs-CRP levels. The purpose of the current study was to investigate the relationship of perceived stress to hs-CRP levels in overweight and obese males.

C. Research Questions

(1) Is there a positive association between hs-CRP levels and psychological stress levels in overweight and obese males?

(2) If there is a positive association between hs-CRP levels and stress, does it apply to both obese and overweight males?

(3) Is there a positive association between hs-CRP levels and stress levels in overweight and obese males using body mass index versus bioelectrical impedance as a measure of overweight and obesity?

D. Significance to Preventive Care

Currently, preventive care practitioners and other health care professionals use LDL cholesterol levels and other risk factors such as diet and exercise practices to screen for cardiovascular risk. However, testing hs-CRP in clinical practice to determine cardiovascular disease risk diagnosis is gaining acceptance (Ridker, Rifai, Rose, Buring & Cook, 2002). The American Heart Association and the Centers for Disease Control and Prevention (2003) have made an official statement of the importance of inflammation markers when evaluating risk for cardiovascular disease. "...it is reasonable to measure hs-CRP as an adjunct to the major risk factors to further assess absolute risk for coronary disease primary prevention" (cf, Pearson, et al, 2003, p. 509). When considering secondary prevention they state, "In patients with stable coronary disease or acute coronary syndromes, hs-CRP measurement may be useful as an independent marker for assessing likelihood of recurrent events, including death, myocardial infarction, or restenosis after percutaneous coronary intervention" (cf, Pearson, et al, 2003, p. 509). Therefore, hs-CRP levels are likely to be given more value in clinical practice in the future.

Healthy People 2010, objective 12-1, emphasizes the need for a 20% reduction of coronary heart disease deaths between 2000 and 2010. In addition, objective 20-9 emphasizes a 13% increase in worksite programs that prevent or reduce employee stress

(Healthy People 2010; 1998). If the present study shows a positive association between hs-CRP levels and stress levels among an already at-risk population of overweight and obese males, further studies can be done to determine if stress reduction is a possible strategy to lower cardiovascular events by way of hs-CRP reduction. If no relationship is found, physiologically different mechanisms may be present that are unique to depression among the obese that should be investigated further.

CHAPTER 2

REVIEW OF LITERATURE

The influence of psychological stress on the body, and its potential relationship to atherosclerosis by way of inflammation in obese men, is a new concept. The relationship between the mind and body is rapidly gaining more attention. The importance of the body working as a whole dynamic system is seen by increased attention and research in the field of psychoneuroimmunology (Dugue, Leppanen, Teppo, Fyhrquist & Grasbeck, 1993; Black, 2003; Padgett & Glaser, 2003). The proposed study attempted to link the areas of stress, inflammation, and obesity with the goal of identifying new areas for cardiovascular disease prevention. In order to understand the proposed link, each area needs to be defined and explained. The chain of events discussed is how psychological stress might influence inflammation (as detected by hs-CRP), in overweight and obese males.

A. Psychological Stress and Body Pathways

Many studies have been done on the effect of psychological stress on the immune system (Dugue, Leppanen, Teppo, Fyhrquist & Grasbeck, 1993; Black, 2003; Padgett & Glaser, 2003). The term stress can be used in a very general and broad sense. However, the cause of stressors can be broken down into external versus internal stressors. External stressors, as perceived by the individual, defined as events such as divorce or death of a loved one, is a type of cognitive sensory stimulus initially processed through the peripheral and central nervous systems. Internal stressors are considered non-cognitive stressors, which include bacterial and viral infections processed by the immune

system first and passed through to the neuroendocrine system (Blalock, 1984). Cognitive stress, the focus of this investigation, has been associated with psychoneuroimmunology (PNI) concepts, clinical research and application (Bauer-Wu, 2002). PNI is a field that studies the multifaceted bi-directional interaction between the immune system and the central nervous system (CNS). Central to this field is the influence of psychosocial factors on physical health (Cohen & Powderly, 2004).

There is a cascade of events that occurs in the body upon exposure to stressful events. This cascade begins in the CNS. The impact of the CNS on the immune system is primarily mediated by changes in the activity of the hypothalamic-pituitary-adrenal (HPA) and sympathetic adrenal-medullary (SAM) axes. As part of the stress response, these systems are activated causing an upregulation in plasma levels of glucocorticoids and catecholamines. Glucocorticoids and catecholamines are classes of immunomodulatory hormones capable of binding to cytoplasmic receptors on leukocytes that alter their function and distribution (Cohen & Powderly, 2004). This summarizes the link between the psychological stress beginning in the CNS and ending in a biochemical effect on the immune system.

Another link that exists occurs between the immune system and other biological systems dealing with cytokines. Immune cells produce cytokines which maintain homeostasis by acting as modulators of the immune system. These cytokines have been shown to communicate with other biological systems such as the HPA. If imbalances or alterations occur in the function of these immunomodulators, illness usually results (Wong, 2002). This demonstrates the bi-directional effect of the immune system

affecting the HPA. Although not the direct focus of this study, this provides background on the influence of the immune system and other systems and mechanisms in the body.

There is an established bi-directional relationship between the stress (mind) and the immune system (body) (Wong, 2002; Cohen & Powderly, 2004; Bauer-Wu, 2002). This link is important to the proposed study because it investigates the possible association between psychological stress and the immunological response of inflammation in individuals (overweight and obese) already at risk of inflammation.

B. High Sensitivity C-Reactive Protein

Hs-CRP is a marker of inflammation, primarily produced in hepatocytes and regulated by interleukin 6 (IL-6), interleukin 1 (IL-1), tumor necrosis factor alpha (TNF-alpha) and other cytokines (Rerranti & Rifai, 2002). Recent studies have shown that elevated CRP is associated with increased risk of atherosclerosis (Ridker, Rifai, Rose, Buring, & Cook, 2002). This classical acute-phase protein is a very sensitive systemic marker of disease with broad clinical uses for monitoring disease status and providing differential diagnosis of disease state.

High sensitivity C-reactive protein is found circulating in the blood of healthy individuals; however, upon situations such as bacterial invasion or injury an acute phase response results which increases hs-CRP levels drastically. Serum concentrations begin rising approximately six hours, peaking approximately 48 hours after only a single stimulus. In the majority of diseases, circulating values of hs-CRP are more accurate when determining current inflammation than other methods such as plasma viscosity and erythrocyte sedimentation rate. This is explained by the even half-life of hs-CRP. Hs-CRP's half-life stays constant around 19 hours under all conditions; therefore, the only

determinant of the plasma concentration is synthesis rate which therefore reflects intensity of the pathological processes stimulating CRP production (Hirschfield & Pepys, 2003). Other than obesity there are no specific physical conditions that correlate with general CRP levels. However, hyperglycemia, metabolic syndrome, chronic infection, weight loss, medication and alcohol consumption, are conditions associated with fluctuations of CRP using the high sensitivity CRP (hs-CRP) test (King, Mainous & Taylor, 2004).

CRP values are a very helpful non-specific biochemical marker of inflammation (Hirschfield & Pepys, 2003). They are not impaired by disease states other than liver failure. Very few drugs reduce CRP values unless they also affect the underlying acute-phase stimulus. This is helpful in the clinical setting because most patients would qualify and benefit from the hs-CRP test with the understanding that their results will be skewed by illness or by being placed on certain medications.

In a review of the literature, Rosenson and Koenig (2003) discuss studies that support the concept that system measures of CRP actually reflect the number of vulnerable atherosclerotic plaques. In addition, they review studies that show elevated CRP (greater or equal to 3 mg/L) levels predict an increased rate of recurrent ischemic episodes, progression to acute myocardial infarction and revascularization procedures when compared to those with lower CRP levels. The American Heart Association and the Centers for Disease Control and Prevention developed a scientific statement on markers of intervention and cardiovascular disease stating that individuals with an LDL cholesterol level below the standard for initiating drug therapy and a Framingham ATP III 10-year score (10%-20%) is an individual that warrants hs-CRP testing (Pearson et al,

2003). The rationale for this recommendation is that people fitting into an intermediate risk category with an elevated hs-CRP level would provide a reason for beginning lipid-lowering therapy (Davidson, 2004). Pai, et al. (2004) showed that elevated levels of certain inflammatory markers, in particular hs-CRP, indicate an increased risk of coronary heart disease. They studied 504 subjects testing for plasma levels of sTNF-R1, sTNF-R2, interleukin-6, and hs-CRP. Among subjects who provided a blood sample and were free of heart disease at baseline, 265 men and 29 women had a nonfatal heart attack or fatal coronary heart disease during six and eight years of follow-up, respectively. Plasma lipid levels had a stronger association with increased risk of CHD than were the other inflammatory markers tested; the level of hs-CRP remained a significant contributor to the prediction of coronary heart disease after controlling for lipid and non-lipid factors.

Further investigation is needed on the specific role that inflammation plays in CAD as determined by hs-CRP. This topic warrants further attention and serious consideration because of its possible impact on diagnosis and prevention of CAD in the clinical setting. However, based on the information that currently exists, hs-CRP testing is recommended upon the discretion of the physician, especially in individuals with stable coronary disease (Pearson et al, 2003). When using the available inflammatory measures, hs-CRP holds the most promise as a possible screening tool for cardiovascular disease. The American Heart Association and Centers for Disease Control and Prevention (2003) issued a statement that hs-CRP testing may be most useful as a means to augment risk assessment in patients who have already been identified at some risk due to other factors such as hyperlipidemia. However, it is clear that further clinical trials are needed to

establish whether elevated hs-CRP itself needs to be screened for and treated when present in isolation (King, Mainous & Taylor, 2004).

C. Stress, the Immune System and Inflammation

The release of catecholamines, glucocorticoids and endogenous opiates are significant in the field of PNI because immune and neuroendocrine cells have receptors for these stress hormones (Bauer-Wu, 2002). In a review of the literature, Kiecolt-Glaser, McGuire, Robles and Glaser (2002) concluded there is sufficient data to deduce immune modulation by psychosocial stressors can lead to actual health changes.

The implications of the association between psychological stress and the immune system span a wide variety of outcomes. For example, immune dysregulation induced by stress can lead to conditions related to aging, osteoporosis, arthritis, type 2 diabetes, some cancers and cardiovascular disease (Kiecolt-Glaser, McGuire, Robles & Glaser, 2002). The literature published on stress has shown many of the same results. The following are examples of such research. Schleifer, Keller, Camerino, Thornton and Stein (1983) studied 15 spouses of women who had advanced breast cancer. They found that when the breast cancer victim died, suppressed immunity followed in the surviving spouse. They specifically tested lymphocyte stimulation responses to phytohemagglutinin, concanavalin A, and pokeweed mitogen. Irwin, Daniels, Smith, Bloom, and Weiner (1987) studied women whose spouses had died and also found suppressed immunity in the surviving spouse, in the form of decreased natural killer cell activity. A dramatic life event that induces psychological stress is experiencing the death of a loved one. In these studies, the surviving spouse experienced suppressed immunity as a result of stress. This

demonstrates that life events not only affect an individual's psychological state but can also open the body to pathogens and other physiological reactions.

The psychological stress experienced by many college students has also shown to produce an immune response. Paik, Toh, Lee, Kim and Lee (2000) studied 42 college students and examined their blood the day before an examination and again at four weeks later. They found an increase, prior to the exam, in Th2 cell-mediated humoral immunity and macrophage activities and a possible decrease in Th1 cell-mediated cellular immunity indicating an activation of the immune system. Specifically, stress caused by the upcoming academic examination increased IL-1 beta, IL-6, and IL-10 and decreased IFN-g production.

Exposure to trauma can result in immune dysregulation in the realm of post-traumatic stress disorder (PTSD). PTSD is associated with inflammatory conditions such as cardiovascular disease, rheumatoid arthritis and inflammatory bowel disorders (Wong, 2002). The studies described above establish an association between psychological stress and the immune system even when different sources of stress are involved.

Psychological stress has also been linked to the susceptibility of infection. Cohen, Tyrrell and Smith (1991) studied psychological stress and the common cold. Subjects were asked to complete questionnaires assessing degrees of psychological stress and 394 healthy subjects were given nasal drops containing one of five respiratory viruses. Twenty-six subjects were used as controls and were given saline nasal drops. The subjects were then quarantined and watched for the development of infection and symptoms. Clinical colds were defined as clinical symptoms in the presence of an infection confirmed by the isolation the virus or presence of increased levels of a virus-

specific antibody titer. The authors concluded that psychological stress was associated, in a dose response manner, with an increased risk of acute infectious respiratory illness. They suggest that the risk was attributable to increased rates of infection rather than to an increased frequency of symptoms after infection.

The association between cognitive stress and the immune system is important for the present study because it links the two areas together. Specifically, the study examined the possible association of psychological stress and the immunological reaction of inflammation as assessed by hs-CRP, in overweight and obese men, who are already at risk for increased hs-CRP levels.

The association between psychological stress and hs-CRP as a marker of inflammation has not been as thoroughly investigated as stress and other forms of immunological response noted above. However, in 2001, Steptoe, Willemsen, Owen, Flower and Mohamed-Ali investigated the influence of acute mental stress on the cardiovascular responses and concentrations of inflammatory cytokines. Their results showed a 56% increase in interleukin 6, a major modulator of hs-CRP, two hours after stressful behavioral tasks (color-word interference and mirror tracing). This suggested that inflammatory cytokines respond to acute mental stress in humans.

Hapauarachchi, Chalmers, Winefield and Blake-Mortimer (2003) measured various markers of psychological stress and correlated them with biological markers such as hs-CRP, homocysteine, salivary immunoglobulin A and oxidative stress in 76 healthy individuals of various weights. They assessed stress and other psychological variables using the General Health Questionnaire (GHQ-12), Copenhagen Burnout Inventory, Perceived Stress Scale, Anger Expression Scale, Nowack's Coping Scale, Occupational

Role Questionnaire, Occupational Strain Questionnaire, Nowack's Social Support Scale and the Warr, Cook, and Wall Scale for job satisfaction. Among their results they showed that psychological stress is associated with pro-inflammatory states as evidenced by increased hs-CRP concentrations. Specifically they found mean differences between the normal (mean=1.09) and severely (mean=3.36) stressed for hs-CRP levels ($p < .05$).

Dugue, Leppaen, Teppo Fyhrquist and Grasbeck (1993) studied whether psychological stress influenced immunobiological functions. Two kinds of stress were studied: the Stroop color conflict test and the thrill of a first time parachute jump. The level of stress indicators cortisol or anti-diuretic hormone rose significantly in both test situations. The concentrations of the cytokines studied did not change. However, the parachute test showed a significant positive correlation between changes in cortisol and hs-CRP. Their results suggest that there is a positive interaction between the endocrine and the immune system in the response to an acute psychological stressful experience.

Melamed, Shirom, Toker, Berliner and Shapira (2004) studied the association of fear of terror with low-grade inflammation among apparently healthy employed adults. They studied 1,153 subjects, of various body weights, assessing terror by measuring the extent to which respondents have deep concerns for personal safety, elevated tension in crowded places, and fear of terror strikes causing harm to one's self or one's family. They concluded that chronic fear of terror is associated with elevated hs-CRP levels (adjusted OR = 1.7, 95% CI=1.2-2.4) suggesting the presence of low-grade inflammation and a potential risk of cardiovascular disease. However, this association was found only in women, not men, suggesting that there may be different inflammatory processes in action between the genders.

Hossain, Latif, and Udin (2005) studied Bangladeshi people to see if hs-CRP is a sensitive marker of an individual experiencing a stressful state in both the mind and body. They studied 55 subjects, ages 18-55, over a one year period. They defined stressful conditions as those who had infection, psychiatric disorders and those who were post surgery. Based on different kinds of stress they divided their experimental group into four subgroups: infection, psychiatric, pre and post surgery. They found a statistically significant rise of hs-CRP levels seen in the infection, psychiatric disorder and post surgery stage groups. They concluded that stressful conditions resulted in an increased synthesis of hs-CRP by liver cells due to hypothalamopituitaryadrenal (HPA) axis hyperactivity and immune mediated inflammation.

C-reactive protein is currently being investigated in order to establish the hs-CRP test as a viable and accurate test for heart disease. Biasucci (2004), reporting on a Centers for Disease Control and Prevention and American Heart Association workshop on markers of inflammation and cardiovascular disease, states that hs-CRP has consistently been found to be independent from other risk factors for cardiovascular disease. Hs-CRP has also been shown to have an incremental value beyond the common risk factors and biochemical markers of risk such as interleukin-6, interleukin-1RA and troponin.

The above studies show that a relationship exists between psychological stress, especially acute stressors, and the immune system, specifically inflammation. Some studies have specifically used hs-CRP testing to detect the presence of an inflammatory immunological response to stress.

D. Depression, Obesity and High Sensitivity CRP

Barth, Schumacher and Hermann-Lingen (2004) conducted a meta-analysis on depression as a risk factor for mortality in patients with coronary heart disease (CHD). They searched English and German databases from 1980 to 2003 for prospective cohort studies and identified 62 publications. They found that depressive symptoms and clinical depression increases mortality in CHD patients. Miller, Freedland, Duntley and Carney (2005) examined depressive symptoms associated with markers of infection and inflammation related to pathogenesis of coronary artery disease. After studying 65 patients recovering from an acute coronary syndrome, they found that patients who had more severe depressive symptoms exhibited higher levels of hs-CRP.

Suarez (2004) studied hs-CRP's association with psychological risk factors of cardiovascular disease in healthy adults. He studied 127 healthy, nonsmoking men and women and found that greater anger and severity of depressive symptoms, separately and in combination with hostility, were significantly associated with increased CRP levels. Furthermore, these associations were independent of potential confounding factors.

Ladwig, et al. (2003) conducted a population-based study of 3,204 men aged 45-74. They investigated the influence of depressive mood on the association of hs-CRP and obesity in healthy men. Obesity, defined as a body mass index greater than 30, was found in 726 individuals (23% of sample). A non-fasting venous blood sample was taken from all participants in a supine resting position and hs-CRP levels were measured using a high-sensitive immuno-radiometric assay (hs-CRP with a range of 0.05-10.0 mg/L). In addition, depressive symptomatology was assessed using a subscale from the von Zerssen affective symptom check list. The sample was stratified into three levels of depressive

mood which revealed a significant association (p=.008) between increased hs-CRP in the obese sample with highest level of depression (n=147, hs-CRP adjusted mean=3.22) when compared to moderate (hs-CRP adjusted means=2.44) and low (hs-CRP adjusted mean=2.43) level depression groups.

The obesity and depression interaction was found to be significant (p=.021). There was no association between depressive mood and concentrations of hs-CRP in the non-obese group. Multivariate analysis confirmed this interaction after adjusting for possible confounders such as smoking, high blood pressure and age.

The present study used the Ladwig, et al. research as a template to investigate a possible association between psychological stress, obesity and increased hs-CRP concentrations. This study attempted to examine stress as the psychological factor as the potential trigger for elevated hs-CRP levels.

E. Inflammation and Atherosclerosis

Inflammatory processes can cause a variety of responses by the host; the responses collectively are referred to as an acute phase response. This response is associated with distinctive metabolic changes in liver protein synthesis. The resulting changes are called acute, mainly because results can be seen within a short period of time (Goldman, 2000). Stress can activate an acute phase response (APR), part of the innate immune inflammatory response which gives evidence that the inflammatory response, is contained within the stress response or that stress can induce an inflammatory response (Black, 2003).

Interestingly, atherosclerosis is now considered an inflammatory disease. The lesions characteristic of atherosclerosis represent a series of specific cellular and

molecular responses that can accurately be described collectively as inflammatory disease. These lesions can lead to ischemia of the heart, brain or extremities resulting in infarction (Ross, 1999).

It is common for lesions to be present throughout a lifetime. The earliest lesions are called fatty streaks. Fatty streaks are pure inflammatory lesions consisting of monocyte-derived macrophages and T lymphocytes, key players in inflammation. In the occasion of excessive and persistent chronic inflammatory processes in the artery, a complicated lesion may result (Ross, 1999). Coronary artery inflammation is involved in all stages of lesion or plaque formation. Lesions can rupture precipitating clotting in patients with myocardial infarction, unstable angina, stenosis, total vessel blockage and sudden death. Unstable lesions have thin, fibrous caps overlying a core rich in lipids containing inflammatory cells that include proteins such as fibrinogen and hs-CRP, which are important determinants of plaque rupture (Rosenson & Koenig, 2003).

Chronic inflammation can result in increased numbers of macrophages and lymphocytes, which immigrate from the blood and have the potential of multiplying in the lesion (Ross, 1999). Activation of these cells leads to the release of many substances including cytokines which modulate the inflammatory marker hs-CRP. Hs-CRP is present in trace concentrations in human plasma; however, when the body experiences inflammatory states, hs-CRP levels rise. The concept that atherosclerosis is an inflammatory disease has clinical significance in that it can be detected easily by the hs-CRP test (Ridker, Fifai, Rose, Buring & Cook, 2002; Ferranti & Rifai, 2002; Ladwig, Marten-Mittag, Lowel, Doring & Koenig, 2003). With atherosclerosis accepted as an

inflammatory disease detected by using the hs-CRP test, the proposed study can incorporate this test as a marker of atherosclerotic risk.

F. Obesity and C-Reactive Protein

Overweight and obesity prevalence continues to dramatically increase in the United States. Currently, more than half of all U.S. adults are overweight or obese. In general, people who become obese do so because of a combination of genetics and a lifestyle which consists of low levels of physical activity and high levels of caloric consumption (Sower, 2003). There have been several studies linking obesity and hs-CRP. Aronson, et al. (2004) found that obesity was one of the major factors associated with elevated hs-CRP in individuals with metabolic syndrome. Metabolic syndrome is a cluster of disorders characterized by abdominal obesity, high blood pressure, high triglyceride level, high fasting glucose level and low HDL cholesterol level. This increased hs-CRP level indicates that obesity may also have an influence on the inflammatory response given that hs-CRP is a biomarker of inflammation.

Weiss, et al. (2004) studied obesity and the metabolic syndrome in adolescents and children. They found that hs-CRP levels rose with increasing obesity, suggesting that biomarkers for cardiovascular disease are already present in this young age group. Warnberg, Moreno, Mesana and Marcos (2004) studied 493 Spanish adolescents who were 13 years old. Although they did not study if adolescent overweight and obesity initiate the development of future disease, they did suggest that obesity states may induce a chronic low-grade inflammatory state as evidenced by a rise in hs-CRP levels. Although specific mechanisms have not been identified, this points to the importance of

maintaining proper body weight in adolescence in the effort to avoid obesity-related disease and chronic inflammation in adulthood.

Pannacciulli, et al. (2001) investigated whether hs-CRP concentrations are influenced by insulin resistance, body composition and body fat distribution in 201 healthy women. They found that hs-CRP was positively correlated with age, body mass index, waist, fasting glucose and insulin, insulin resistance, fat free and fat mass. This study showed there is an independent relationship between central fat accumulation and insulin resistance with hs-CRP plasma levels. Greenfield, et al. (2004) studied obesity as an important determinant of baseline serum hs-CRP concentrations in monozygotic twins, independent of genetic influences. They studied 194 healthy female twins and found that hs-CRP was strongly related to total central abdominal obesity, blood pressure and lipid levels, independent of genetic factors.

Khaodhiar, Ling, Blackburn and Bistrian (2004) examined the correlation of inflammatory markers such as tumor necrosis factor-alpha (TNF), soluble TNF receptor II (sTNF-RII) and, interleukin 6 (IL-6) with BMI in nonobese, obese and morbidly obese individuals. Their study supported idea that obesity represents an inflammatory state. They found that in morbidly obese individuals only, there was a correlation of IL-6 and hs-CRP with BMI, particularly in males. This suggests that IL-6 may be secreted in an endocrine manner in proportion to the expansion of fat mass, specifically in the abdominal region. In addition, this increase corresponded with an increase in hepatic production of hs-CRP. Lee and Prately (2005) also studied the evolving role of inflammation on obesity. They state that it is now clear that the adipocyte is an active participant in the generation of the inflammatory state experienced in obese individuals.

Adipocytes secrete a variety of cytokines such as IL-6 which promotes inflammation. The authors state that an improved understanding of the role of adipose tissue and the characteristic activation of inflammatory pathways could suggest possible novel treatments and prevention strategies, such as fat reduction methods, aimed at reducing obesity-associated morbidities and mortality.

Vozarova, Weyer, Hanson, Tataranni, Bogardus and Pratley (2001) studied circulating interleukin-6 (IL-6) in relation to adiposity, insulin action and insulin secretion. Adiposity was measured by dual energy x-ray absorptiometry (DXA), a high speed fan beam scanner which measures body composition. Results showed that IL-6, a key modulator in hs-CRP production, was positively related to adiposity, suggesting that hs-CRP production may be increased by way of IL-6 because of increased fat. Furthermore, Laimer, et al. (2005) studied the effect of weight loss on nontraditional cardiovascular risk markers such as hs-CRP, interleukin-6 (IL-6) and matrix metalloproteinase-9 (MMP-9), in middle-aged morbidly obese women. They studied 45 morbidly obese women before gastric banding and one year post surgical treatment. They concluded that weight loss is associated with a pronounced decrease in nontraditional cardiovascular risk markers such as hs-CRP, indicating beneficial effects of weight loss on cardiovascular risk.

The above studies show an association between adiposity and hs-CRP, indicating obesity is related to the body's inflammatory immune response. The present study examined how stress and obesity might interact to increase hs-CRP concentrations in the blood.

G. Conclusion

In conclusion, there is evidence that a relationship exists between acute stress and inflammation. There is also an established relationship between inflammation and elevated hs-CRP as well as inflammation, atherosclerosis and increased hs-CRP levels in the obese. No published studies have examined psychological stress and hs-CRP levels in overweight and obese males. If an association is observed it may lead to a further investigation of psychological stress as an intervention area for the prevention of CAD, especially among overweight and obese males.

CHAPTER 3

METHOD

A. Study Design

This study was a correlational or cross sectional study that measured the degree of linear relationship between two factors measured within defined groups (Morgan, 1996). The defined group was overweight and obese males and the two factors were psychological stress and hs-CRP level.

B. Subjects

Subjects were healthy males aged 20-35 recruited from Loma Linda University Adventist Health Sciences Center (LLUAHSC) entities. Subjects were recruited via flyers. Flyers were placed in the Loma Linda University Medical Center, each of the seven schools that make up Loma Linda University (LLU) as well as the Drayson Center, Del Webb Library and Student Services. (See Appendix A.) Mass e-mails were sent to all students in LLU School of Public Health and to all LLU medical residents. In addition, after each subject was tested they were asked to refer anyone who fit the criteria for participation in the study. Flyers were also carried to many departments in LLUASHC and subjects were personally invited to participate. Thirty-two subjects were recruited to LLU Center for Health Promotion (CHP) for testing. Paperwork was resubmitted to the Institutional Review Board for approval of recruitment and testing at other locations. Upon approval, testing was set up at LLU Drayson Center, the university's fitness facility. Twenty-nine subjects were recruited and tested on site at

the Drayson Center and given a bottle of water as a token of appreciation for participation.

C. Inclusion/Exclusion Criteria

Subjects were included in the study if they were male and had a body mass index (BMI) of 25 or above, classifying them as overweight or obese. Age was limited to those ages 20-35 in order to reduce the influence of age as a confounding factor. Participants were excluded if they were smokers or had any injuries or viral or bacterial infections within the past 30 days. These states increase hs-CRP levels because of their inflammatory effect on the body. Participants were also excluded if they had taken any form of anti-inflammatory medication within 48 hours of testing. Participants were excluded if they had taken medications such as, but not limited to, aspirin, ibuprofen, naproxen and prescription NSAIDS within 48 hours. The use of these medications decreases hs-CRP levels because of their anti-inflammatory properties.

D. Instrumentation

The primary variables assessed are listed below.

1. Perceived Stress Scale

The Perceived Stress Scale (PSS) is a widely used psychological tool which measures the perception of stress. The PSS was used to measure the degree to which life's situations are appraised as stressful. Each item was designed to assess how individuals find their lives to be unpredictable, uncontrollable and overloaded. The scale contains 10 questions using Likert-type response questions (Cohen, Tyrrell & Smith, 1993). "The PSS has adequate internal and test-retest reliability and is correlated in the expected manner with a range of self-report and behavioral criteria ($r=.65$, $p<.05$)"

(Cohen, Kmarack & Mermelstein, 1983, p. 392). (See Appendix B.)

2. Blood Pressure

Blood pressure was taken using a standard blood pressure cuff and scale. Blood pressure was taken on the left arm in a relaxed sitting position.

3. Body Mass Index/Body Fat

Height and weight were assessed using a regular dual reading eye-level physician scale in order to calculate body mass index (BMI). Body fat percentage was assessed using an Omron HBF-306, a handheld body fat analyzer. This bioimpedance machine provides results in seven seconds and calculated BMI upon entering an individual's height and weight. Bioimpedance is a noninvasive method used to evaluate body composition. A small electric current is passed through the hands and results are based on the principal that body fat conducts electricity different than body muscle.

4. High Sensitivity CRP Testing

After blood samples were collected from participants, hs-CRP testing was performed at Loma Linda University Medical Center's clinical laboratory using high sensitivity CRP testing (hs-CRP) to determine CRP levels. This technology uses two monoclonal antibodies specific to CRP and forms a unique two-site combination for the development of a fast and sensitive CRP assay (Advanced ImmunoChemical Inc.)

E. Procedures

Participants were invited to the exercise laboratory in Evans Hall and the Martinson and Fritz room in the Drayson Center located on the Loma Linda University campus. Subjects were given a verbal and written explanation of the study as part of the informed consent process. (See Appendix C.) This included an explanation of the

benefits and risks of participation along with an explanation of the study. The subjects were also told they would receive a telephone call informing them about their hs-CRP results approximately two weeks after testing. Subjects were informed that if their test results showed elevated hs-CRP they should to contact their primary care physician or student health services for further care and evaluation.

1. Informed Consent Document

After subjects had signed the informed consent document (ICD), their height and weight were measured in order to calculate their body mass index (BMI). Height (feet and inches) and weight (pounds) were measured without shoes and articles in subjects pockets. Height and weight measurements were then entered into the Omron HBF-360, a hand-held fat analyzer, and BMI and body fat percentage were calculated and assessed at the same time. If participants had a BMI of 25 or over they were included in the study. If they had a BMI of less than 25 they were excused.

2. Screening

Subjects were then asked to fill out a screening form (see Appendix C) in order to assess if they met study criteria. If they did not meet the criteria they were excused; if they met criteria, subjects were given the 10 item Perceived Stress Scale. This stress scale took approximately five minutes to complete.

3. Blood Draw

Lastly, subjects had their blood drawn for the purpose of hs-CRP testing. The hs-CRP test is not affected by food consumption or time of day; therefore, blood draws occurred at any time. The blood draws were taken in a sitting position using antecubital venipuncture by a licensed phlebotomist. The site depended on the individual

and which vein was more visible for puncture. A tourniquet was placed around the upper arm to restrict blood flow through the vein, then antiseptic was applied to the site. This technique causes the veins below the tourniquet to distend and fill with blood allowing blood to be collected in an air tight vial. The tourniquet was then removed and the puncture site was covered to stop any bleeding after the sample was taken.

Possible risks that can occur when puncturing a vein included excessive bleeding, bruising, hematoma and numerous punctures in order to find a vein. In addition, some individuals may become lightheaded or faint if they are uncomfortable with the sight of blood, pain or needles. Subjects were informed of these possible risks both orally and in writing in the informed consent document (ICD) and were given the opportunity to withdraw their participation at any time. To reduce the risk of bruising, subjects were advised to keep pressure on the site for 10 minutes after the needle was withdrawn. A cotton ball held tight with tape was used to help subjects keep pressure on the site. Thirty-two of the blood draws were completed in the Center for Health Promotion, a medical clinic capable of handling fainting or any other emergencies that may occur beyond which the primary investigator or the phlebotomist can handle. Twenty-nine of the blood draws were performed at the Drayson Center which is less than half a mile from Loma Linda University Medical Center, a trauma 3 facility. The facility is capable of handling major medical emergencies.

The blood samples were taken within an hour to Loma Linda University Medical Center's clinical laboratory for analysis. The hs-CRP test employs a method called nephelometry. This method uses specialized instruments to measure the movement of

particles in solution or turbidity (http://health/quantitative-immunoglobulins-nephep://health.allrefer.com/ lometry-info.html, 2004).

At the end of the session, subjects were given a form with their recorded height, weight, BMI, body fat percentage and blood pressure. In addition, the form contained a brief description of hs-CRP risk categories. (See Appendix E.) There are three hs-CRP risk categories for developing cardiovascular disease: low risk (< 1.0 mg/L), average risk (between 1.0 – 3.0 mg/L), or high risk (>3.0 mg/L) (Centers for Disease Control and Prenvention & American Heart Association, 2003). The author verbally explained these risk categories to subjects at the time of the blood draw as well as over the phone when subjects were informed of their results. If hs-CRP values were elevated, subjects were strongly encouraged to contact their primary care physician or student health services for further evaluation and care.

F. Statistical Analysis

Descriptives such as frequencies, means, outliers and normal distribution were determined for all study variables. Correlations of hs-CRP levels, stress, body fat percentage and BMI values were done to evaluate if a linear relationship existed among these variables. The sample size of 60 was based on a power analysis of 80% and the hypothesis that stress is linearly associated with hs-CRP level, based on an alpha of 0.05.

CHAPTER 4

PUBLISHABLE PAPER

Psychological Stress and High Sensitivity C-Reactive Protein Levels in Overweight and Obese Men

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Submitted to Journal of Behavioral Medicine
Planned date of submission: July 2005

Psychological Stress and High Sensitivity C-Reactive Protein Levels in Overweight and Obese Men

In one study, depression was positively associated with high sensitivity C-reactive protein (hs-CRP) in 726 obese men (Ladwig, et al., 2003), but little is known about how chronic psychological stress might be associated with the same inflammatory processes. This study investigated the influence of perceived stress of daily life and whether it is associated with hs-CRP in overweight and obese males. Sixty-one men participated in this study which included the 10-item Perceived Stress Scale (PSS) and hs-CRP testing. There was no association between perceived stress and hs-CRP levels (r=.06, p=.65). Hs-CRP levels were significantly associated with body mass index (r=.43, p=.001) and body fat percentage (r=.49, p=.0001).

Over the past few years, high sensitivity C-reactive protein (hs-CRP) has received much attention for its role in atherosclerosis (Pearson, Mensah, Alexander, Anderson, Cannon, Criqui, Fadl, Fortmann, Hong, Myers, Rifiai, Smith, Tubert, Tracy & Vinicor, 2003; Rosenson & Koenig, 2003; Plutzky, 2001). This association was validated on January 28, 2003, when the American Heart Association and the Centers for Disease Control and Prevention published a joint scientific statement in *Circulation*, stating that from a pathological viewpoint, all stages of atherosclerosis, including initiation, growth, and complication of atherosclerotic plaque, might now be considered to be an inflammatory issue (Pearson, et al., 2003). Subsequently, there are now many studies

investigating the magnitude of the role hs-CRP plays in atherosclerosis and the accuracy with which hs-CRP levels can predict future cardiac events (Arenillas, Alvarez-Sabin, Molina, Chacon, Montaner, Rovira, Ibarra, & Quinanna, 2003; Folsom, Pankow, Tracy, Arnett, Peacoch, Hong, Djousse & Eckfeldt, 2001).

The association between hs-CRP and atherosclerosis has spawned research into factors that may amplify this association. Psychological variables such as depression, hostility and fear have emerged as possible factors that lead to increased hs-CRP levels (Melamed, Shirom, Toker,Berliner & Shapira, 2004; Miller, Feedland, Duntley & Carney, 2005; Barth, Schumacher & Herrmann-Ligen, 2004). Suarez (2004) showed that greater anger and severity of depressive symptoms, separately and in combination with hostility, were significantly associated with elevations in hs-CRP. Melamed, et al. (2004) showed elevated hs-CRP levels in women who experienced chronic fear of terror, suggesting the presence of low-grade inflammation and a potential risk of cardiovascular disease. Depression has also been shown to elevate hs-CRP levels (Miller, et al., 2005). This mounting evidence shows that psychological factors may indeed have an inflammatory effect.

Biological factors such as obesity also play a clear role in hs-CRP levels. Adipocytes or fat cells are active participants in the generation of the inflammatory state of obesity (Lee & Pratley, 2005). In addition, obesity using the measurement of body mass index (BMI) is positively related to hs-CRP (Khaodhiar, Ling, Blackburn & Bistrian, 2004; Laimer, 2005).

One study found that acute stressful conditions, defined as subjects experiencing infection and psychiatric disorder in a post surgery stage, showed increased synthesis of

CRP by liver cells due to hypothalamopituitaradrenal (HPA) axis hyperactivity and immune mediated inflammation (Hossain, Latif & Uddin, 2005). This indicates a linkage between the mind and body regarding acute stress states and increases in inflammation as indicated by hs-CRP levels.

Ladwig, et al. (2003) studied the effect of depression on hs-CRP levels. They found that hs-CRP concentrations were higher among males with depressive mood, but only for those in the obese category. The authors used a subscalse from the von Zerssen Affective Check List to assess depressive symptomology. The obese sample (n=726) was stratified into three levels of depressive mood which revealed a significant association (p=.008) between increased hs-CRP in those with the highest tertile of depression (hs-CRP adjusted mean=3.22) compared to moderate (hs-CRP adjusted means=2.44) and low (hs-CRP adjusted mean=2.43) level depression groups. According to the CDC and American Heart Association (2003), statement hs-CRP risk categores are as follows: <1.0=low risk, 1.0-3.0=average risk and >3.0 is high risk. Ladwig, et al. state that psychological factors such as depression, and biological factors such as obesity, when present together, can influence inflammatory processes associated with atherosclerosis. The question then arises that if depression, a serious clinical mental state, produces inflammatory processes in obese males, whether chronic psychological stress might also produce an inflammatory response in a similar population.

No research has been published on whether hs-CRP levels increase due to chronic stresses of daily life as perceived by the individual. The present study investigates the relationship of perceived stress to hs-CRP levels in overweight and obese men.

METHOD

Participants

Subjects were overweight or obese but otherwise healthy males aged 20-35. They were recruited by word-of-mouth, flyers and personal invitation at a private health science campus in Southern California. Subjects were included in the study if they were male and had a body mass index (BMI) of 25 or above, classifying them as overweight or obese. The sample was limited to those ages 20-35 in order to eliminate age as a confounding factor. Participants were excluded if they were smokers or had any injuries or viral/bacterial infections within the past 30 days. These states elevate hs-CRP levels due to increased inflammation (Dovi, Szpaderska & DiPietro, 2004; Sumi, Satoh, Ishikawa & Sekizawa, 2004). Participants were excluded if they had taken any form of anti-inflammatory medication within 48 hours of testing, such as aspirin, ibuprofen, naproxen and prescription NSAIDS. Main study variables included hs-CRP level, psychological stress, body mass index (BMI), body fat percentage and blood pressure. Exercise, perceived health status, religious attendance and sleep were also measured.

Procedures

Subjects were given a verbal and written explanation of the study as part of an informed consent process. After subjects had signed the informed consent document, their height and weight were measured in order to calculate their body mass index (BMI). Height (feet and inches) and weight (pounds) were measured without shoes and articles in subjects' pockets. Height and weight measurements were then entered into the Omron HBF-360, a hand-held fat analyzer, and BMI and body fat percentage were calculated

and assessed at the same time. If participants had a BMI of 25 or over they were included in the study. If they had a BMI of less than 25 they were excused.

Subjects were then asked to fill out a screening form in order to assess if they met the rest of the study criteria. If they did not meet criteria they were excused; if they met criteria subjects were given a 10 item Perceived Stress Scale (PSS). Lastly, subjects had their blood drawn for the purpose of hs-CRP testing. The hs-CRP test is not affected by food consumption or time of day; therefore, blood draws occurred at any time. Blood draws were taken in a sitting position using antecubital venipuncture by a licensed phlebotomist. These procedures were approved by the Institutional Review Board of Loma Linda University.

Psychosocial Measures

Psychological stress was assessed by the Perceived Stress Scale (PSS), a widely used psychological tool which measures the perception of stress. The PSS was used to measure the degree to which life's situations are appraised as stressful. Each item is designed to assess how individuals find their lives to be unpredictable, uncontrollable and overloaded. The scale contains 10 items using Likert-type response format (Cohen, Tyrrell & Smith, 1993). "The PSS has adequate internal and test-retest reliability and is correlated in the expected manner with a range of self-report and behavioral criteria ($r=.65$, $p<.05$)" (Cohen, Kmarack & Mermelstein, 1983, p. 392).

Inflammatory Risk Marker

Hs-CRP testing was performed at Loma Linda University Medical Center's clinical laboratory using hs-CRP to determine blood levels. This technology uses two

monoclonal antibodies specific to CRP and forms a unique two-site combination for the development of a fast and sensitive CRP assay (Advanced ImmunoChemical, Inc.)

RESULTS

Descriptive Statistics

Sixty-one subjects were tested in total with a mean age of 26.9 (SD=4.2). The mean PSS score was 14.08 (SD = 6.7), with a range of 1 to 33, with 40 being the highest score possible. (See Table 1) Using body mass index categories of 25-29.9 as overweight and 30 or above as obese, 28 (45.9%) were classified as overweight and 33 (54.1%) were classified as obese. However, using body fat percentage categories, 33 (54%) were classified as overweight (17%-25% body fat) and 28 (45.3%) were obese (>25% body fat), with the result that nine subjects were classified differently depending on whether BMI or body fat percentage was used. BMI values ranged from 25.5 to 48.2 with a mean of 32.0 (SD=5.4). Body fat percentage values ranged from 12.7% to 38.2% with a mean of 24.3 (SD=6.8). High sensitivity CRP levels ranged from .30 to 5.54, with 11 subjects (18.3%) being in the high risk category (3 or above). (See Table 1) Comparisons on study variables between overweight and obese categories, as determined by body fat percentage, are shown in Table 1. After the Bonferonni adjustment for multiple comparisons, four out of the six associations were no longer significant (p=.005).

Descriptive statistics for study variables between overweight and obese groups showed PSS scores were higher in obese (mean=12.8) compared to overweight

Table 1 Descriptive Statistics for Study Variables Overall and Between Overweight and Obese Groups*

Variable	Total (n=61) Mean (SD) or (%)	Overweight (n=33) Mean (SD) or (%)	Obese (n=28) Mean (SD) or (%)	p-value
Age	26.9 (4.2)	25.7 (3.9)	28.0 (4.1)	**.03**
BMI	32.0 (5.4)	28.1 (1.8)	36.5 (4.7)	**<.0001***
Systolic BP	115.9 (10.4)	113.0 (10.0)	118.3 (10.7)	**.05**
Diastolic BP	73.0 (9.2)	70.4 (9.8)	75.2 (9.2)	**.05**
hs-CRP	2.3 (1.8)	1.1 (1.1)	2.3 (1.8)	**.002***
Perceived stress score	14.1 (6.7)	12.8 (6.3)	15.2 (6.9)	.16
Hours of sleep	6.8 (1.1)	6.9 (1.3)	6.7 (0.8)	.32
Education				.48
High school	4 (6.6)	1 (3.0)	3 (10.7)	
College	28 (45.9)	16 (48.5)	12 (42.9)	
Post graduate	29 (47.5)	16 (48.5)	13 (46.4)	
Religious attendance				.71
Not at all	14 (23.0)	9 (27.3)	5 (17.9)	
Once per month	9 (14.8)	6 (18.2)	3 (10.7)	
2-3 /month	4 (6.6)	2 (6.1)	2 (7.1)	
1/week	24 (39.3)	12 (36.4)	12 (42.9)	
>1/week	10 (16.4)	4 (12.1)	6 (21.4)	
Health status				**.02**
Fair	15 (24.6)	5 (15.2)	10 (35.7)	
Good	25 (41.0)	11 (33.3)	14 (50.0)	
Very Good	14 (23.0)	11 (33.3)	3 (10.7)	
Excellent	7 (11.5)	6 (18.2)	1 (3.6)	
Physical Activity				.211
Rarely or never	4 (6.6)	3 (10.7)	4 (6.6)	
<1/week	3 (4.9)	3 (10.7)	3 (4.9)	
1/week	9 (14.8)	4 (14.3)	9 (14.8)	
2-3/week	29 (47.5)	13 (46.4)	29 (47.5)	
4-5/week	10 (16.4)	4 (14.3)	10 (16.4)	
6+/week	6 (9.8)	1 (3.6)	6 (9.8)	

**Groups based on body fat % where 17%-25% was overweight and >25% were classified as obese. **P-values reach Bonferroni-adjusted criterion of .005.*

(mean=15.2) men, but this difference was not significant. In the overweight group, 48.5% reported they were in fair or good health as compared to 85.7% of the obese group (p=.02). Those in the obese group were older, and had higher systolic and diastolic blood pressure, hs-CRP levels and BMIs. After the Bonferoni adjustment (p=.005) only BMI and hs-CRP levels remained significantly different between overweight and obese groups, which is consistent with other studies.

Perceived stress scores showed no significant association with hs-CRP levels (r=.060, p=.647). (See Table 2) There was also no significant association between PSS and hs-CRP when broken down into overweight (r= - .01, p=.96) and obese (r=.04, p=.98) categories. Spearman Rho correlations were used because the distribution of hs-CRP levels were not normal and because several of the other study variables such as health status were on an ordinal scale. Hs-CRP levels were then transformed using square root and log 10 calculations, which yielded a more normal distribution. However, no association between hs-CRP values and PSS (using Pearson correlation) was found even after the transformation. Body mass index was significantly associated with body fat percentage (r=.90), systolic blood pressure (r=.37), hs-CRP (r=.43), and perceived stress score (r=27). Body fat percentage was significantly associated with diastolic blood pressure (r=.39) and hs-CRP (r=.49). Health status was negatively correlated with body mass index (r=-.38) and body fat (r=-.36).

There were no significant associations for hours of sleep and BMI, body fat percentage, hs-CRP levels and education level, religious service attendance and hours of sleep.

Table 2 Spearman Rho Correlations Among Key Study Variables (N=61)

Variable	BMI	Body Fat %	Blood Pressure (Systolic)	Blood Pressure (Diastolic)	hs-CRP	Perceived Stress Score
Body Fat %	**.906 (p=.000001)**					
Blood Pressure (Systolic)	**.325 (p=.011)**	**.293 (p=.022)**				
Blood Pressure (Diastolic)	**.353 (p=.005)**	**.346 (p=.006)**	**.498 (p=.00004)**			
hs-CRP	**.546 (p=.00001)**	**.592 (p=.000001)**	-.037 (p=.777)	.015 (p=.422)		
Perceived Stress Score	.233 (p=.070)	.149 (p=.253)	.035 (p=.788)	-.041 (p=.752)	.060 (p=.647)	
Health Status	**-.375 (p=.003)**	**-.361 (p=.004)**	.007 (p=.955)	*-.278 (p=.030)*	*-.361 (.004)*	-.218 (.091)

**After Bonferroni adjustment, p-value was .005.*

DISCUSSION

Similar to past research, both BMI and body fat percentage were significantly positively associated with hs-CRP level, and CRP showed a slightly stronger associated with body fat percentage (r=.592) than BMI (r=.546). This may indicate that the measurement of body fat is a better predictor of increased levels of hs-CRP than the measurement of height and weight alone. Interestingly, BMI showed borderline significance and association to PSS (r=.233), whereas body fat percentage (r=.149) did not. BMI and body fat percentage were also significantly associated with both systolic

and diastolic blood pressures, as found in other research. However, both blood pressures showed a stronger association with body fat percentage than BMI.

As expected, body mass index and body fat percentage were highly associated. However, it is important to note that nine out of 61 subjects (14.7%) were classified depending on composition measurements used. When using BMI, seven subjects were classified as overweight whereas using body fat they were considered obese and two were classified as obese but as overweight using body fat. Therefore, use of body mass index should not to replace body fat testing when establishing overweight or obese classification. Body mass index measures only height and weight; therefore, individuals who have high muscle content leading to increased weight would be classified as obese according to BMI measurements (Peterson, 2002).

This study did not show a linear association between chronic stresses of daily life and hs-CRP levels ($r = .06$), even after transforming the non-normally distributed hs-CRP values. If a relationship exists, it is likely a small effect size, which would only be detected with more subjects and perhaps a more sensitive assessment of stress. The lack of association in this study compared to other studies on stress and hs-CRP may be due to the lower sample size, few subjects with hs-CRP values above 3, younger age and the type of psychological state being tested in this study. For example, Ladwig et al. (2003) showed there was an association between depressive mood among 726 obese subjects men ages 45-74, whereas the current study investigated chronic stress of daily life among 61 overweight and obese men ages 20-35. The differences concerning age criteria and psychological states of depression versus chronic stresses of daily life may account for the differences between results of the present study versus the Ladwig et al. study.

This study also found a negative association between body fat percentage and perceived health status (r= -.361). Health status was not correlated with hs-CRP, when controlling for body fat percentage r=-.08 (p=.54), but body fat percentage and health status were correlated (r=. 30, p=.02), when controlling for hs-CRP. This indicates the strength of the association of health status is with body fat percentage and not hs-CRP.

Some studies have investigated the correlation between the presence of acute psychological stressors and hs-CRP levels (Dugue, Leppaen, Teppo, Fyhrquist & Grasbeck, 1993; Melamed, Shirom, Toker, Berliner & Shapira, 2004). Positive associations were found, but the psychological states examined were fear of terror and the thrill of a first time parachute jump, rather than of chronic stresses of daily life. These acute states are likely different than the chronic stresses of daily life tested in this study and may influence the inflammatory response in different ways.

Possible explanations for this difference may be due to variations in coping styles of each individual. The way a person perceives and manages stress may have an impact on how the body responds to various stressors. Individuals with positive ways of coping are associated with low pro-oxidant states as indicated by lower CRP levels (Hapuarachchi, Chalmers, Winefield & Blake-Mortimer, 2003). Another possible explanation could be that the severity of the stressor may impact the body differently. The intensity of the stressor may influence the amount of inflammation that occurs, but this is yet to be examine directly.

Exercise status may also have played a role in the results of this study. Twenty-nine (47.5%) subjects were recruited from the Drayson Center, a university gymnasium and health facility. Forty-five (74%) subjects reported that they participated in vigorous

exercise two or more days a week. These subjects were involved in exercise to a greater extent than the general population, which could have influenced their response to stress, how they coped with stress, or the inflammatory processes in the body. Although a good distribution of stress values was found, it may suggest that these individuals have more leisure time that would deem them less stressed or these individuals may have an effective way to cope with stress. Exercise may counteract the negative effects of stress on hs-CRP levels, if such effects exist.

The finding that chronic psychological stresses of daily life are not associated with hs-CRP levels in overweight and obese males points to a need of further investigations into the link between acute stress and depressive mood and how they differ from stresses of daily life, types of stressors or coping mechanisms in relation to hs-CRP levels. Depressive mood, acute stress and obesity states may have possible positive, negative or even synergistic relationships with hs-CRP that are yet to be discovered. The specific mechanisms and dose response of this mind-body connection are still largely unknown.

This study confirmed a strong positive association of both BMI and body fat percentage with hs-CRP but showed no relationship of chronic stress of daily life on hs-CRP levels in overweight or obese males.

Strengths and Limitations

This study was based on sound measurements. The methodology included validated and reliable measures of psychological stress, body mass index, body fat percentage and hs-CRP. In addition, blood testing was completed within two hours of each blood draw, which reduced possible errors caused by standing blood.

The possibility of low power (sample size) did not allow for the detection of small effect sizes or associations, if they exist, between the Perceived Stress Scale and hs-CRP; however, small effect sizes may not be clinically significant. Clearly strong associations of BMI, body fat and hs-CRP were detected in this sample and those associations are clinically important. In addition, the use of a cross-sectional design does not allow for a cause and effect relationship to be determined, if an association exists.

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CHAPTER 5

DISCUSSION

A. Discussion

Past studies show associations between acute stress situations and hs-CRP levels in the blood (Dugue, Leppaen, Teppo, Fyhrquist & Grasbeck, 1993; Melamed, Shirom, Toker, Berliner & Shapira, 2004). One study also found a positive relationship between depressive mood and hs-CRP levels in obese men (Ladwig et al., 2003). These studies contribute to the theory that the mind can have an influence on the body thereby causing disease states. The present study was conducted to add to the growing amount of research that furthers the understanding of this mind-body relationship.

This study found no linear association between chronic stresses of daily life as measured by the Perceived Stress Scale (PSS) and high sensitivity C-reactive protein in this sample of overweight or obese males. Spearman rho correlations were determined because of the non-normal distribution of hs-CRP values which where highly positively skewed. After log 10 and square root transformations which normalized the hs-CRP distribution values, there was still no association between PSS score and hs-CRP.

The studies that have shown a correlation between stress and hs-CRP have primarily measured acute psychological stress situations such as the thrill of a first time parachute jump (Dugue, Leppaen, Teppo, Fyhrquist & Grasbeck, 1993) and fear of terror of harm to themselves or their families due to terrorist strikes (Melamed, Shirom, Toker, Berliner & Shapira, 2004). This dissertation study measured chronic stress, which may have different mechanisms at work in its influence on inflammation as compared to

chronic stress. Acute stressors are situations where stress occurs severely during a short period of time as compared to chronic stress, which can be less severe but occurs over a long period of time creating different reactions in the body.

Differences between acute and chronic stressors need to be further investigated to uncover the variations in inflammatory responses. Differences such as intensity and duration of stressors may be important factors that cause these differences. Differences in inflammatory responses may also be caused by variations in coping styles (Marcenaro, Prete, et al., 1999). The way one perceives stress and manages stress may possibly change the way one's body reacts to psychological factors.

Exercise status may have also played a role in the results of this study. Twenty-nine (47.5%) subjects were recruited from the Drayson Center, which is a gymnasium and health facility. Forty-five (73.7%) of subject reported exercising two times a week or more, which could influence their stress level or CRP production. King, Baumann, O'Sullivan, Wilcox and Castro (2002) conducted a 12-month randomized controlled trial in 100 women aged 49 to 82. The objective of their study was to determine the health and quality of life effects of moderate-intensity exercise among older women family caregivers. They concluded that there are benefits from initiating a regular moderate-intensity exercise program in terms of reductions in stress-induced cardiovascular reactivity and improvements in rated sleep. In addition, Kondo, Nomura, Nakaya, Ito and Ohguro (2005) studied the association of hs-CRP with aerobic exercise capacity, maximum oxygen uptake and insulin resistance in 50 healthy subjects. They used a multi-step treadmill exercise test to attain the maximum oxygen uptake. Their results suggest that the development of exercise habits increases the maximum oxygen uptake,

thereby reducing the inflammatory marker hs-CRP. Therefore, the relationship of exercise on stress can be beneficial and may have influenced the inflammatory response in individuals who participated in a regular exercise program.

The results of this study confirmed an association between increased hs-CRP levels and overweight and obese individuals. The linkage confirms the seriousness of overweight and obese states. These states have shown to produce many disease processes such as diabetes, reproductive complications and urinary problems. The issue of increased inflammation as indicated by elevated hs-CRP levels also shows the relationship of overweight and obese states with heart disease.

Eleven subjects (18%) had hs-CRP levels of three or above which is considered to be high risk according to the Centers for Health Promotion and Prevention and the American Heart Association (2003). Reducing BMI and body fat even by a few points move an individual from the obese to the overweight category, and can significantly improve an individuals hs-CRP levels as a result. The mean hs-CRP levels for the overweight category were 1.1 (SD=1.1) and the obese were 2.3 (SD=1.8) (p=.002). The obese subjects were not in the high risk category of hs-CRP levels which is probably due to the younger age of the study population, however, values are double that of those in the overweight category.

The lack of a relationship between chronic stresses of daily life and hs-CRP levels in overweight and obese males points to the need for further research on psychological stress and inflammation. Differences between acute stressors and chronic stressors, depressive mood and chronic stress, and mechanisms that regulate each need to be investigated.

B. Strengths and Limitations

This study was based on a sound instruments. The methodology included validated measures of psychological stress (Cohen, Tyrrell & Smith, 1993), body mass index, body fat percentage and hs-CRP. The use of tools and methods that have been validated and used for years, increases the likelihood that variables were measured accurately and appropriately. The Perceived Stress Scale has been used in research that links perceived stress with bacterial vaginosis during pregnancy, inflammatory bowel disease and avoidance in pseudoseizures (Culhane, et al., 2001; Frances, Baker & Appleton, 1999; Sewitch, et al., 2001). The PSS has been used as the tool to assess perceived stress under differing situations and has proven to be a valid instument. The hs-CRP test is also a validated measurement used in the clinical setting and recommended by the Centers for Disease Control and Prevention and the American Heart Association (2003) as an assessment tool to evaluate heart disease risk. Errors were also reduced because measurements were taken and recorded immediately. For example, blood testing was completed within two hours of each blood draw, which reduced possible errors caused by standing blood and errors in paperwork.

The present study was also based on logical assumptions of similar relationships between acute stress and depression with increased hs-CRP levels. The investigation of a correlation between chronic stress and increased hs-CRP levels in overweight and obese males was based on previous research conducted on acute stressors and depression. Acute stressors (Dugue, Leppaen, Teppo, Fyhrquist & Grasbeck, 1993; Melamed, Shirom, Toker, Berliner & Shapira, 2004) and depression (Ladwig, et al., 2003) were

related to increased hs-CRP levels; therefore, a possible correlation between chronic stress and increased hs-CRP was deemed reasonable.

When setting up the study design, a medium effect size was assumed, based on previous literature. Therefore, a sample size of 60 subjects was calculated based on a power analysis of 80%, alpha=0.05, and an expected correlation between CRP and stress levels of at least r=.30. However, the low power used in the study does not allow for the detection of small effect sizes such as those found. Using Spearman's rho, assuming a small effect size of .06, the study would have had to test 1,050 subjects to reach significance. Using Pearson's correlation coefficient for a small effect size of .10, 400 subjects would have been needed.

The time of testing differed for each subject, which may have affected hs-CRP levels. CRP possesses an even half-life and stays constant around 19 hours under all conditions (Hirschfield & Pepys, 2003); therefore, it is unlikely that the time of day influenced the results. However, times of collection varied from 8 am to 10 pm, and time of blood draw may prove to be a factor in further research.

Another limitation of the study was the narrow range of hs-CRP levels found, which ranged from .30 to 5.54 with 11 (18.3%) being high risk. This study had lower hs-CRP values compared to the Ladwig et al. study, which had a broader range of hs-CRP levels and was probably due to differences in age which may have allowed for the detection. Another factor that limited the study was the restriction of age. Subjects were included in the study only if they were 20 to 35 years old, whereas Ladwig et al. (2003) studied men ages 45-74 (x=58.3). This criterion limited the generalization of results to

only this younger age group, which may not exhibit enough of an inflammatory response to stressors.

Another possible limitation to the study may have been the use of the Perceived Stress Scale as the tool for assessing psychological stress. It was a tool created in the 1980s, and has been validated with coefficient alphas in a .84-.86 range. It has been used many times by its author, Cohen, and by others, and consistently found to be associated with inflammatory bowel disease and immune-inflammatory changes. Maes, et al. (1999) used the PSS to test 38 university students to determine that academic examination of stress created changes in the distribution of peripheral blood mononuclear cells, indicating immune activation, which is assumed to be orchestrated by stress-induced production of cytokines. In addition, Sewitch, et al. (2001) used the PSS with 200 patients and found that strategies aimed at improving social support can have a positive impact on psychological distress, which improved health outcomes for these patients with inflammatory bowel disease. The PSS was also used by Song, et al. (1999), who studied 38 university students and concluded that psychological stress induces immune-inflammatory changes pointing toward complex regulatory responses in IL-6 signaling, a decreased anti-inflammatory capacity of blood serum and interactions with T cell and monocyte activation. The use of the PSS in these studies show that the instrument is an appropriate tool because of its reliability and validity in similar research on biopsychosocial outcomes including immune parameters.

The main limitation of this study was the use of a cross-sectional design. This type of study design cannot demonstrate causation, if an association of PSS and hs-CRP exists. Nevertheless, it provides preliminary evidence that stress, as measured by the

Perceived Stress Scale, does not appear to be directly related to hs-CRP in young, healthy, overweight and obese males.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

A. Conclusions

High sensitivity CRP has become a popular topic in clinical practice over the past few years. Currently hs-CRP testing is not a standard practice in the clinical setting (AHA & CDCP, 2003). The strength of hs-CRP testing in contributing to the diagnosis of heart disease and predicting future cardiac events remains to be seen. However, current studies show that hs-CRP is a beneficial test to run separately or in conjunction with total cholesterol levels (AHA & CDCP, 2003). The use of this inflammatory marker as an accepted clinical tool is promising. One of the purposes of this study was to determine if a psychological factor, stress might be associated with this inflammatory marker in an attempt to strengthen the argument that hs-CRP testing is vital in the prevention and detection of heart disease. The following is a list of study conclusions:

1. Body mass index and body fat percentage are highly correlated with elevated hs-CRP levels.
2. Chronic stress of daily life is not linearly associated with hs-CRP levels in young, otherwise healthy overweight and obese men.
3. Health status is negatively associated with body fat, BMI and hs-CRP; however, most of this association is explained by body composition rather than hs-CRP.

B. Research Recommendations

An association between chronic psychological stress and CRP was not found in this study. (See Appendix F) Therefore, further investigation needs to be done on this topic using a larger sample size in order to uncover possible smaller associations if they exist. Chronic psychological stress did not produce an inflammatory response in either overweight or obese males, indicating possible mechanism differences between chronic and acute stress. The following questions are posed for future research in this area:

1. Does the severity of the negative psychological state, such as stress, affect the amount and type of inflammation in the body?
2. Does length of time (acute versus chronic) of negative psychological state, such as stress, affect the amount and type of inflammation in the body?
3. Does exercise status affect stress and inflammation levels in the overweight and obese? In addition, does exercise affect coping mechanisms thereby influencing inflammatory responses?
4. What is unique about the association of hs-CRP to depressive mood and acute psychological stress that is different from chronic stress as measured by PSS?
5. How much does coping style influence hs-CRP levels in overweight and obese men? Does the way an individual perceives and manages stress influence the bodily response to stressful situations?
6. Is there a difference between males and females concerning psychological stress and hs-CRP levels?

C. Practice Recommendations

Although there was no association found between chronic stresses of daily life and hs-CRP levels in young overweight and obese men, there are applications to clinical practice that can be recommended. Obesity clearly must be targeted as a condition that increases inflammation and in turn, heart disease. Obesity is associated with many disease processes (Neilson & Schneider, 2005); however, the hs-CRP test can be used to uncover increased risk of heart disease or future coronary events above and beyond traditional risk factors, especially among overweight and obese individuals. Laimer, et al. (2005), showed that weight loss is associated with a pronounced decrease in non-traditional cardiovascular risk markers such as hs-CRP. Therefore, health education can then be used to discuss exercise and nutrition strategies to decrease body fat with the ultimate goal of decreasing risk of heart disease.

This study also showed that body fat percentage influences an individual's perceived health status. A negative association between body fat percentage and perceived health status was found (r=-.361). Health status was not correlated with hs-CRP, when controlling for body fat percentage r=-.08 (p=.54), but body fat percentage and health status were correlated (r=. 30, p=.02), when controlling for hs-CRP. The implications of these results show that self-perception of health status and body fat can be targeted in health education. Therefore, issues concerning body fat arise again as areas that need to be targeted by preventive care efforts.

The association between the mind and the body is a powerful relationship. The mechanisms that govern these reactions are still largely unknown. As research and knowledge increase, more aspects of these mechanisms will be understood. Studies such

as this provide information concerning the mind-body linkage and contribute to the quest for understanding this complex relationship. The present study attempted to uncover another psychological state that might be associated with increased hs-CRP levels in overweight and obese males. However, the results of this study found no association, pointing to the need for further investigation of other unknown mechanisms and characteristics involved between psychological states and the inflammatory system.

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Appendix A

Recruitment Documents

WANT TO CONTRIBUTE TO MEDICAL SCIENCE?

FREE FREE

By joining this study you will receive:

- **FREE** body mass index and body fat assessment
- **FREE** blood pressure testing
- **FREE** high sensitivity C-reactive protein (CRP) blood test assessing atherosclerotic risk

Requirements for study:

- Healthy **male**
- **20-35** years old
- **ABOVE** normal weight

Study procedures will involve:

- About **30 minutes** of your time
- A **blood draw** of about 1 tsp of blood
- Height, weight, blood pressure and body fat assessment
- Completing a **short survey**

*RESULTS WILL BE **COMPLETELY CONFIDENTIAL***

(Loma Linda University School of Public Health Dissertation Study)

FOR MORE INFORMATION CALL: **(909) 799-6613** *or*
email:olivialm@verizon.net

olivialm@ verizon.net (909) 799-6613	olivialm@ verizon.net (909) 799-6613	olivialm@ verizon.net (909) 799-6613	olivialm@ verizon.net (909) 799-6613	olivialm@ verizon.net (909) 799-6613	olivialm@ verizon.net (909) 799-6613	olivialm@ verizon.net (909) 799-6613	olivialm@ verizon.net (909) 799-6613	olivialm@ verizon.net (909) 799-6613

Paycheck Stuffer

Research Project: C-reactive Protein
You are invited to participate in a dissertation research project with the School of Public Health. The project is investigating the relationship of psychological stress to C-reactive protein (CRP) levels in overweight and obese men. Participants will receive free CRP, blood pressure and body composition testing. C-reactive protein testing is associated with the detection of atherosclerosis. Participants must be healthy males between 20 to 35 years of age, above normal weight, and have not taken anti-inflammatory medication within 48 hours of testing. Testing session will last approximately 30 minutes. For more information and if you are interested, contact Olivia Moses of the school of public health at (909) 799-6613.

Telephone Script

Hello, thank you for your interest in our research study. C-reactive protein is a biomarker for inflammation and can be used as an indicator for atherosclerosis or atherosclerotic risk. We are looking for people that fit certain criteria. Can I ask you a few questions? What is your height and weight? Are you on any medications such as Ibuprofen, Naproxen, prescription NSAIDS or aspirin?

If participant fits criteria: I would be delighted if you would be willing to participate in this study. When you come to the Center for Health Promotion located on the corner of Anderson and Stewart Street you will be given material describing the study and also be given a chance to ask any questions. You will be given a one page questionnaire with 10 questions and you will have your blood pressure taken. You will then have your height and weight measured and your body fat tested. Next, you will have your blood drawn. CRP testing only requires one teaspoon of blood, therefore, it is a quick procedure. In addition, CRP testing is not associated with food intake so please feel free to eat before testing. The whole session should take approximately 30 minutes. Do you have any questions about the study or its procedures? Can I set you up with a time slot for you to come to the Center for Health Promotion? If you know of anyone else that would fit the criteria and would be willing to participate please have them contact me for more information. I am very thankful to you for your participation in this study and your part in helping me obtain my doctoral degree.

If participant does not fit criteria: Unfortunately, for this purposes of this particular study you do not fit the criteria. However, if you are interested in C-reactive protein and its role in atherosclerosis you are welcome to come to the Center for Health Promotion during the times of testing to receive a free informational hand-out. Thank you for calling and your willingness to participate.

Appendix B

Perceived Stress Scale

Perceived Stress Scale

The questions in this scale ask you about your feelings and thoughts **during the last month**. In each case, you will be asked to indicate by circling how often you felt or thought a certain way.

Name_____ Date_____

Age_____ Gender (Circle): M F Other_____

0 = Never 1 = Almost Never 2 = Sometime 3 = Fairly Often 4 = Very Often

1. In the last month, how often have you been upset because of something that happened unexpectedly?.....0 1 2 3 4

2. In the last month, how often have you felt that you were unable to control the important things in your life?.....0 1 2 3 4

3. In the last month, how often have you felt nervous or "stressed"?....0 1 2 3 4

4. In the last month, how often have you felt confident about your ability to handle your personal problems?.....0 1 2 3 4

5. In the last month, how often have you felt that things were going your way?.....0 1 2 3 4

6. In the last month, how often have you found that you could not cope with all the things that you had to do?.....0 1 2 3 4

7. In the last month, how often have you been able to control irritations in your life?.....0 1 2 3 4

8. In the last month, how often have you felt that you were on top of things?.....0 1 2 3 4

9. In the last month, how often have you been angered because of things that were outside of your control?.....0 1 2 3 4

10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?.....0 1 2 3 4

Appendix C

Screening Form

CRP Study Questionnaire

Name:_____
(please print clearly)

1. **In the last month have you experienced or been diagnosed with any of the following conditions?** *Check all that apply.*

☐A Acute bacterial infection
☐B Angina
☐C Cancer(s)
☐D Common cold
☐E Gingivitis (gum disease)
☐F Heart attack
☐G Major trauma
☐H Mucosal infection (e.g., bronchitis, cystitis)
☐I Pancreatitis
☐J Rheumatic disease
☐K Seizures
☐L Stroke
☐M Systemic vasculitis
☐N Other *(please specify)*_____

2. **How often do you engage in vigorous physical activity for at least 20 minutes?** *Check one.*

☐0 Rarely or never
☐1 Less than once per week
☐2 Once per week
☐3 2-3 times per week
☐4 4-5 times per week
☐5 6+ times per week

3. **How often do you engage in mild or moderate physical activity for at least 20 minutes?** *Check one.*

☐0 Rarely or never
☐1 Less than once per week
☐2 Once per week
☐3 2-3 times per week
☐4 4-5 times per week
☐5 6+ times per week

4. **What medications are you currently taking (within the past 48 hours)?** *Check all that apply.*

☐A Aspirin
☐B Ibuprofen (e.g., Advil, Motrin)
☐C Naproxen (e.g., Anaprox, Naprosyn)
☐D Prescription NSAIDS (e.g., Celebrex, Vioyx)
☐E Other over-the-counter medications *(please specify)*_____
☐F Other prescription medications *(please specify)*_____

5. **What is the highest level of education you have achieved?** *Check one.*

☐1 Below high school ☐2 High school ☐3 College ☐4 Post-graduate

6. **How often have you attended religious services in the <u>past month</u>?** *Check one.*

☐0 Not at all
☐1 Once per month
☐2 2-3 times per week
☐3 Once per week
☐4 More than once per week
☐5 Every day or almost every day

7. **On average, how many hours of sleep do you get in a 24-hour period? _____ hours**

8. **How would you currently rate your health?** *Check one.*

☐1 Poor ☐2 Fair ☐3 Good ☐4 Very good ☐5 Excellent

9. **What is your age?** _____ years

Investigator Use Only ***Date of testing:*** ____/____/____

Height: _____ inches Weight:_____ lbs BMI:_____

Body Fat %:_____ Blood Pressure: _____ PSS Score:_____

Appendix D

IRB Approval Forms

INSTITUTIONAL REVIEW BOARD

OSR # 54251

Initial Approval Notice - Expedited Review

OFFICE OF SPONSORED RESEARCH • 11188 Anderson Street • Loma Linda, CA 92350
(909) 558-4531 (voice) • (909) 558-0131 (fax)

To: **Marshak, Helen H**
Department: **Health Promotion & Education**
Protocol: *The relationship of psychological stress to C-reactive protein levels in overweight and obese men*

This study was reviewed and approved administratively on behalf of the IRB. This decision includes the following determinations:

1. Risk to research subjects: **Minimal**
2. Approval period begins **10/13/2004** and ends **10/12/2005.**
3. Stipulations of approval are: **<None Specified>**

Consent Form

If a written consent form is required, approval will be indicated by the affixed IRB approval stamp. This now becomes your official consent form for the dates specified and should be used as a master for making the necessary copies.

Adverse Events / Protocol Changes

The IRB should be notified in writing of any modifications to the approved research protocol. All adverse effects, anticipated or not, should be reported to the IRB: serious events should be reported within seven days; all others within 15 days.

Protocol Review

To assure uninterrupted approval of this project, you are required to complete and return a status report at least two weeks prior to the approval end-date indicated above. (See http://research.llu.edu - select "IRB Tools for Investigators", then "Research Report Form.") In addition to requesting a renewal, you may also use the Research Report Form to close the study.

Records

All records relating to this project, including signed consent forms, must be kept on file for three years following completion of the study.

Please note the PI's name and the OSR number assigned your IRB application (as indicated above) on any future communications with the IRB about this project. Direct all communications to the IRB c/o the Office of Sponsored Research.

Thank you for your cooperation in LLU's shared responsibility for the ethical use of human subjects in research

Signature of IRB Chair/Designee: R Z Rigsby

LOMA LINDA UNIVERSITY

School of Public Health

Loma Linda, California 92350
(909) 558-4546
FAX: (909) 558-4087

Title of Study: The relationship of psychological stress to c-reactive protein (hs-CRP) levels in men

Purpose of Study:

The purpose of this study is to examine the relationship of stress to C-reactive protein (CRP) levels in males who are above normal weight. CRP level is an indicator of inflammation, which is linked to coronary artery disease. This study is being used as the dissertation project for Olivia Moses in partial fulfillment of her Doctor of Public Health degree in the Loma Linda University School of Public Health.

Description and Procedures:

If you agree to participate, the following procedures will be performed in the order listed:

(1) Complete a short questionnaire on physical activity, medication use, and other lifestyle factors.
(2) Psychological stress levels will be assessed by completing a short, 10-item questionnaire.
(3) Height and weight will be measured in order to calculate your body mass index (BMI).
(4) Body fat percentage will be assessed using a hand-held bioimpedence machine. This machine sends a small electric current through the hands and results are based on the principal that body fat conducts electricity different than body muscle.
(5) Approximately 1 teaspoon of blood will be drawn from the vein in your arm. This will be analyzed in the following week to determine your CRP level. You will receive the results of this test within two weeks of your participation, via a telephone call from Olivia Moses. If your CRP level is at or above the level determined to be high risk, you will be referred to your primary care physician or student health services for a second CRP test and follow-up.

These procedures will take about 30 minutes.

Risks:

The blood draw may produce discomfort, pain, fainting or feeling lightheaded, infection, bruising, or swelling at the site. A certified phlebotomist will perform the blood draw using a sterile needle and standard blood draw techniques, and apply pressure to the site to reduce bruising. There is no known risk involved with bioimpedence machine used to determine your body fat percentage. The committee at Loma Linda University that reviews human studies (Institutional Review Board) has determined that participation in this study poses *minimal* risk.

Benefits:

By participating in this research, you will help contribute to a scientific understanding of the potential relationship of stress to CRP levels, a marker for coronary artery disease. You will receive the results of the CRP blood test, body mass index calculation, and body fat testing.

Confidentiality:

All information gathered will be used for this study only, to increase knowledge of the effects of stress on CRP levels. Once all subjects have received their CRP blood test results, names and other identifying information will be stripped from the data in order to protect the confidentiality of results.

Participation and Withdrawal:

Your participation in this research project is completely voluntary. You have the right at ANY time to withdraw from this research study.

Contact Persons:

If you have any questions please feel free to ask them at any time. Please contact Olivia Moses at (909) 799-6613 or Dr. Helen Hopp Marshak at (909) 558-4741 in the Health Promotion and Education Department in the Loma Linda University School of Public Health. If you wish to contact an impartial third party not associated with this study regarding any questions or complaints you may have about the study, please contact the Office of Patient Relations, Loma Linda University Medical Center, Loma Linda, CA 92350, phone (909) 558-4547.

Informed Consent Statement:

"I have read the contents of the consent form and have listened to the verbal explanation given by the investigator. My questions concerning this study have been answered to my satisfaction. I hereby give voluntary consent to participate in this study. Signing this consent document does not waive my rights nor does it release the investigators, institution or sponsors from their responsibilities. I may call Dr. Helen Hopp Marshak during routine office hours at (909) 558-4741if I have additional questions or concerns."

"I have been given a copy of this consent form."

Sign Name Date

Witness Signature Date

"I have reviewed the contents of this consent form with the person signing above. I have explained potential risks and benefits of the study."

Investigator Signature Phone: (909) 799-6613 Date

Appendix E

CRP Handouts

Inflammation, Heart Disease and Stroke: The Role of C-Reactive Protein

❖ How does inflammation relate to heart disease and stroke risk?

"Inflammation" is the process by which the body responds to injury. Laboratory evidence and findings from clinical and population studies suggest that inflammation is important in atherosclerosis (ath"er-o-skleh-RO'sis). This is the process in which fatty deposits build up in the lining of arteries.

C-reactive protein (CRP) is one of the acute phase proteins that increase during systemic inflammation. It's been suggested that testing CRP levels in the blood may be a new way to assess cardiovascular disease risk. A high sensitivity assay for CRP test (hs-CRP) is now widely available.

The American Association and the Centers for Disease Control and Prevention recently published a joint scientific statement about using inflammatory markers in clinical and public health practice. This statement was developed after systematically reviewing the evidence of association between inflammatory markers (mainly CRP) and coronary heart disease and stroke.

❖ What's the role of CRP in predicting recurrent cardiovascular and stroke events?

A growing number of studies have examined whether hs-CRP can predict recurrent cardiovascular disease and stroke and death in different settings. High levels of hs-CRP consistently predict new coronary events in patients with unstable angina and acute myocardial infarction (heart attack). Higher hs-CRP levels also are associated with lower survival rate of these people. Many studies suggested that after adjusting for other prognostic factors, hs-CRP was still useful as a risk predictor.

Recent studies also suggest that higher levels of hs-CRP may increase the risk that an artery will reclose after it's been opened by balloon angioplasty. High levels of hs-CRP in the blood seem to predict prognosis and recurrent events in patients with stroke and peripheral arterial disease.

❖ What's the role of hs-CRP in predicting new cardiovascular events?

Most studies show that the higher the hs-CRP levels, the higher the risk of developing heart attack. In fact, scientific studies have found that the risk for heart attack in people in the upper third of hs-CRP levels is twice that of those whose hs-CRP is in the lower third. These prospective studies include men, women and the elderly. Recent studies also found an association between sudden cardiac death, peripheral arterial disease and hs-CRP. However not all of the established cardiovascular risk factors were controlled for when the association was examined. The true independent association between hs-CRP and new cardiovascular events hasn't yet been established.

❖ What is the normal range of hs-CRP level?

1. If hs-CRP level is lower than 1.0 mg/L, a person has a low risk of developing cardiovascular disease.
2. If hs-CRP is between 1.0 and 3.0 mg/L, a person has an average risk.
3. **If hs-CRP is higher than 3.0 mg/L, a person is at high risk.**
4. **If, after repeated testing, patients have persistently unexplained, markedly elevated hs-CRP (greater than 10.0 mg/L), other evaluation should be considered to exclude noncardiovascular causes.**

www.americanheart.org

If you fall into categories described in numbers 3 or 4 above please contact your primary care physician immediately for further evaluation.

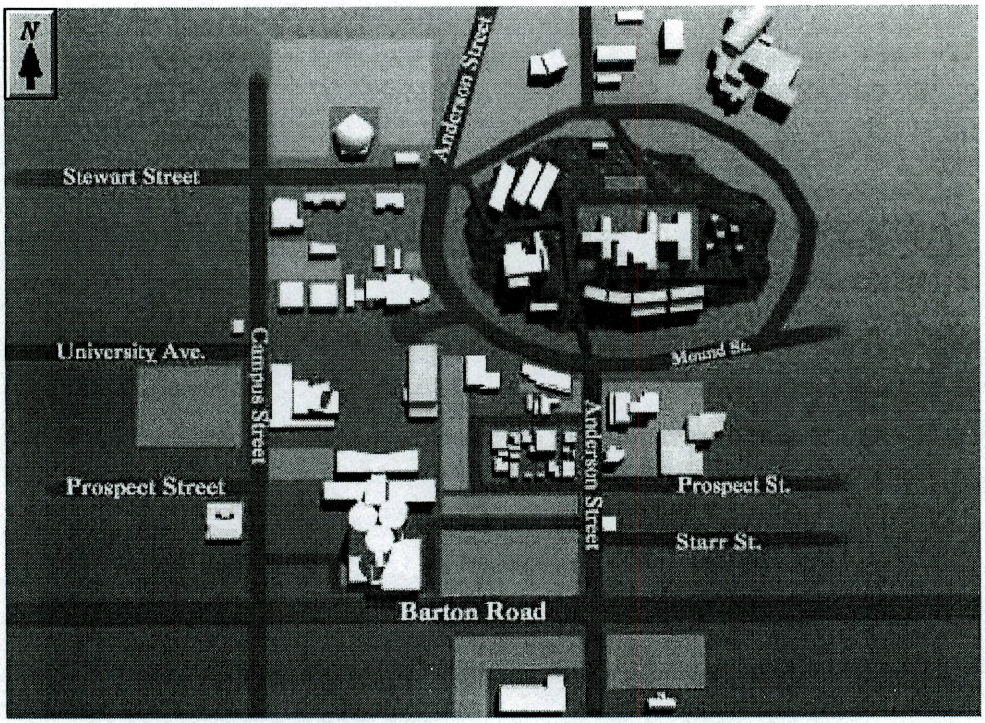
Height:_____

Weight:_____
Body Mass Index:_____
Blood Pressure:_____
Body Fat %:_____

Research Project: C-reactive Protein
You are invited to participate in a dissertation research project with the School of Public Health. The project is investigating the relationship of psychological stress to C-reactive protein (CRP) levels in overweight and obese men. **Participants will receive FREE CRP, blood pressure and body composition testing.** C-reactive protein testing is associated with the detection of atherosclerosis. Participants must be healthy males between 20 to 35 years of age, above normal weight, and have not taken anti-inflammatory medication within 48 hours of testing. Testing session will last approximately 15 minutes. For more information and if you are interested, contact Olivia Moses of the school of public health at (909) 799-6613.

Location: Center for Health Promotion (Student Health in Evans Hall) on the Corner of Stewart and Anderson St.

Appointment:



Appendix F

Scatter Plot of PSS Scores and High Sensitiviy CRP Levels

Scatter Plot of PSS Scores and hs-CRP Levels